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ORIGINAL ARTICLE

Phosphodiesterase 5 inhibitors attenuate renal tubular apoptosis after partial unilateral ureteral obstruction: An experimental study

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Abstract The aim of the present study was to evaluate the effects of phosphodiesterase 5 inhibitors on renal tubular apoptosis and also on expressions of endothelial and inducible nitric oxide synthases (eNOS and iNOS) in the ipsilateral kidney after partial unilateral ureteral obstruction (PUUO) in a rat model. Forty Wistar albino rats were divided into five groups. In Groups 1–4, left experimental PUUO was created. Sildenafil, vardenafil, and tadalafil were administrated to the rats of Groups 2–4, respectively. The pills were orally given to the rats for 30 days. Group 5 was defined as sham. After 30 days, all rats were sacrificed, and nephrectomy was performed. The renal specimens were examined histopathologically. Left hydroureteronephrosis was observed in Groups 1–4. Mean apoptotic cell count and eNOS and iNOS levels were significantly increased in Group 1 when compared with the other groups. The rats in Groups 2–4 showed significantly decreased apoptotic cell count and eNOS and iNOS values in the renal tubular tissue in accordance with Group 1 ($p < 0.05$). There were significant differences in apoptotic cell counts between sildenafil group and the other two study groups. The sildenafil group demonstrated lesser apoptotic cell count than the vardenafil ($p = 0.021$) and tadalafil ($p = 0.009$) groups. PUUO increases the renal tubular apoptosis and elevates NOS concentrations in renal tubular tissue after PUUO. Phosphodiesterase 5 inhibitors have a protective effect against the tubular apoptosis.

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Introduction

Partial ureteral obstruction (PUUO) is one of the most common problems in urological practice. It has been demonstrated that urinary tract obstruction induces progressive apoptosis of both renal tubular and interstitial cells [1]. Tubular cell apoptosis is known as a major factor for the progressive renal tissue loss in obstructive uropathy.

Nitric oxide (NO) is shown to be produced in acute unilateral ureter obstruction and is known to modify renal hemodynamics in the early phase of the obstruction [2]. This substance functions as an antifibrotic factor in the chronic phase of UUO [3]. NO synthase (NOS), the enzyme responsible for the production of NO, has three major isoforms, namely, neuronal, endothelial, and inducible NOS. Although endothelial NOS (eNOS) is Ca^{2+} dependent and is expressed in many tissues, including testes, inducible NOS (iNOS) is Ca^{2+} independent and is induced in tissues after exposure to inflammatory cytokines or ischemia [4].

Sildenafil citrate, vardenafil HCl, and tadalafil are widely used as primary treatment methods of erectile dysfunction (ED). These are phosphodiesterase (PDE) 5 inhibitors and enhance cyclic guanosine monophosphate and NO-mediated vasodilatation with resulting improvement of ED [5]. Although these drugs had been developed for ED, they became one of the most commonly prescribed pharmaceuticals. The relationship between PDE5 inhibitors and apoptosis was also reported. Sildenafil has been recently shown to have a strong protective effect against apoptosis through a NO signaling pathway [6]. The aim of the present study was to evaluate the effects of sildenafil citrate, vardenafil HCl, and tadalafil on renal tubular apoptosis and also on expressions of eNOS and iNOS in rat PUUO model.

Patients and methods

The experimental study was carried out after obtaining the approval of the local Ethics Committee. Forty Wistar albino rats (220—250 g) were enrolled. All surgical interventions were performed under sterile conditions by the same surgical team at the same period and environment. The rats were randomly assigned into five experimental groups. The groups were classified as follows—Group 1 ($n = 8$): PUUO; Group 2 ($n = 8$): PUUO + sildenafil tablet (Viagra, 1 mg/d; Pfizer, NY, USA); Group 3 ($n = 8$): PUUO + vardenafil tablet (Levitra, 0.5 mg/d; Bayer, Leverkusen, Germany); Group 4 ($n = 8$): PUUO + tadalafil tablet (Cialis, 10 mg/72 h; Lilly, Indianapolis, Indiana, USA); and Group 5 ($n = 8$): sham.

The pills were diluted with 10-cc distilled water to form a homogeneous solution and administered to rats for 30 days, orally. The approximate dosages of PDE5 inhibitors, which have been reported previously, were applied [7,8]. Group 5 was defined as sham.

Animal preparation and surgical procedures

After the starvation period of 12 hours, ceftriaxone (20 mg/kg, intramuscularly) was administered as a prophylactic dose 1 hour before the procedure. The rats were placed in supine position after general anesthesia (ketamine, 50 mg/kg, intramuscularly). The abdominal skin was cleaned and shaved. Laparotomy was performed, and the left ureter was isolated. In Groups 1—4, experimental PUUO was created by placing an intravenous catheter into the ureter lumen and by ligating the ureter and catheter together by using 4—0 silk suture. By ligating over the catheter, the ureters had been narrowed surgically. The catheter was then removed cautiously as to not injure the ureter, and the surgical incision was closed. In sham group, ureters were just manipulated.

The rats were housed in individual cages in a temperature (22—24°C), humidity (50—55%), and light/dark cycle controlled room (12/12 h of light and dark) after they had recovered from the general anesthesia. They had free access to food and water, and water was changed daily. They were observed to urinate normally on the same day and feed normally during 30 days.

After 30 days, all rats were sacrificed by an excessive dose of pentobarbital sodium (100 mg/kg, intraperitoneally), and unilateral nephrectomy was carried out. The nephrectomy materials of all the groups were placed separately in 10% formalin.

Histopathological evaluation

All renal specimens were kept at 4°C and then embedded in paraffin blocks. The specimens were then deparaffinized, rehydrated, and sectioned at 5 μm. The sections were microwave pretreated in 10 mM citrate buffer for 20 minutes and were cooled at room temperature for 20 minutes. Afterwards, the sections were washed with buffer solution for 10 minutes. Renal apoptosis was evaluated by using the Apoptosis Protease Activating Factor (Apaf-1) kit (Lab Vision Corp., Neomarkers, CA, USA) for color development. iNOS and eNOS expressions were evaluated by using iNOS Ab-1 and eNOS Ab-1 antibodies (Lab Vision Corp.), respectively. For each group, the number of apoptotic cells and expressions of eNOS and iNOS were calculated by counting positive stained cells in a randomized tissue section with 40× magnification.

Statistical analysis

The statistical data were analyzed using Statistical Package for the Social Sciences (SPSS, Chicago, IL, USA) Version 13.0 for Windows. All values were expressed as mean ± standard deviation. The differences among the groups were compared by one-way analysis of variance and post hoc Tukey tests. A p value less than 0.05 was considered to indicate statistical significance.

Results

Left hydroureteronephrosis was observed in Groups 1—4. Mortality was not observed either in the preoperative period or in the postoperative 30 days. The complications, such as infection, allergic reaction, urination problems, and urinary retention, were not observed in any of the groups. Microscopic findings were used to compare the groups.

PUUO caused significant increases in tubular cell apoptosis, eNOS, and iNOS. Mean apoptotic cell count (Fig. 1) and eNOS and iNOS levels were significantly increased in Group 1 according to the other groups (Table 1).

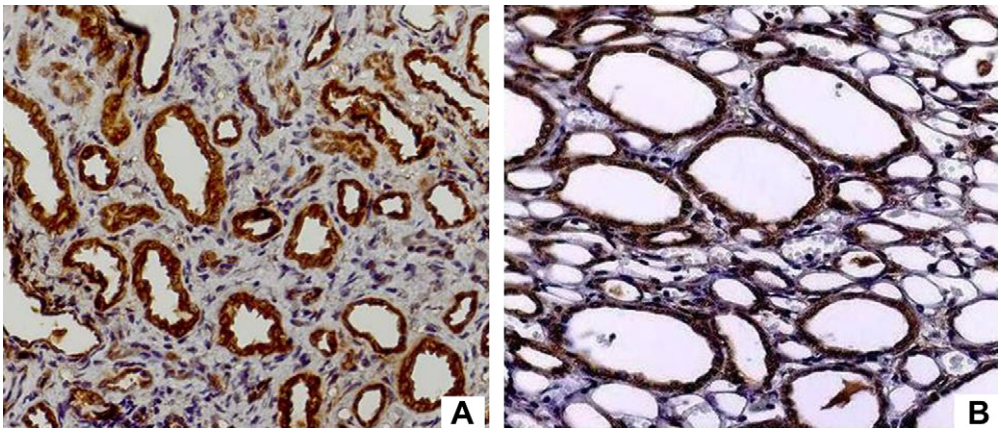

Figure 1. (A) In partial ureteral obstruction (PUUO) group (Group 1), apoptotic cells are markedly increased in renal tubular tissue (immunohistochemical stained method) (40×). (B) The difference of apoptotic cells in PUUO + sildenafil group (Group 2).

In Groups 2–4, the mean values of apoptotic cell count, eNOS, and iNOS were significantly decreased (Fig. 2) when compared with those of Group 1 ($p < 0.05$) (Table 1).

There were significant differences between sildenafil group and the other PDE5 inhibitor–applied groups in terms of mean apoptotic cell count. The sildenafil group demonstrated less apoptotic cell expression than the vardenafil ($p = 0.021$) and tadalafil ($p = 0.009$) groups. However, this difference was not observed in eNOS and iNOS expressions.

Discussion

Tubulointerstitial fibrosis and apoptosis are the two major pathological pathways in obstructive nephropathy. Apoptosis and necrosis occur after ischemia–reperfusion injury [9]. Apoptosis, caused by low-grade injury insufficient to lead to necrosis, has been described in tubules after PUUO and renal ischemia [10,11]. Tubular cell apoptosis is an early event that occurs before the onset of frank fibrosis. As we know from the literature, mechanical stretching of tubular cells provides a major stimulus for apoptosis [12].

The mechanism of the pathophysiological changes in PUUO is not well known. Renal blood flow impairment, intrapelvic pressure elevation, and vasoactive and inflammatory mediators are some of the known factors in pathophysiology of renal obstructive parenchymal injury [13]. Additionally, reactive oxygen species are thought to play an important role in tubulointerstitial inflammation associated with obstructive nephropathy [10,14]. Previous studies have highlighted the importance of apoptosis after complete ureteral obstruction and PUUO. A well-established model of partial unilateral ureteral obstruction in the weanling rat kidney was used to investigate the role of renal tubular apoptosis during hydronephrotic state associated with partial obstruction. The result of this study indicates that apoptosis occurs during the PUUO in a manner similar to that observed in complete ureteral obstruction [15].

The expression of iNOS in the kidney occurs spontaneously [16,17]. The source of NO and its production have not been well characterized. Moreover, the role of NO in renal injury still remains controversial. Both proapoptotic and antiapoptotic effects of NO have been demonstrated so far [18]. NO can be either toxic or protective, depending on the situation. In the kidney, many types of cells are capable of secreting NO. However, the changes in intrarenal pressure accompanying UUO could result in the activation of tubular NOS [19]. Miyajima et al. [20] have previously confirmed NO production in renal tubular epithelial cells exposed to mechanical stretch. In this study, the expressions of eNOS and iNOS in the renal tubular system were significantly increased in PUUO group in accordance with the sham group.

PDE5 is shown to be widely distributed in the smooth muscles of vessels and internal organs, the striated

Table 1 Comparison of the groups according to histopathological parameters

Histopathological parameters	Group 1 (partial ureteral obstruction)	Group 2 (sildenafil)	Group 3 (vardenafil)	Group 4 (tadalafil)	Group 5 (sham)
Apoptotic cells[a]	14.12 ± 0.99	5.37 ± 0.74	6.62 ± 0.51	6.75 ± 0.88	6.87 ± 0.64
Endothelial nitric oxide synthase[b]	2.62 ± 0.51	1.75 ± 0.46	1.62 ± 0.51	1.75 ± 0.46	1.87 ± 0.35
Inducible nitric oxide synthase[c]	2.75 ± 0.46	1.87 ± 0.35	1.75 ± 0.46	1.87 ± 0.64	2

[a] $p = 0.0001$ for Group 1 *vs.* Group 2; $p = 0.0001$ for Group 1 *vs.* Group 3; $p = 0.0001$ for Group 1 *vs.* Group 4; $p = 0.021$ for Group 2 *vs.* Group 3; and $p = 0.009$ for Group 2 *vs.* Group 4.

[b] $p = 0.005$ for Group 1 *vs.* Group 2; $p = 0.001$ for Group 1 *vs.* Group 3; $p = 0.005$ for Group 1 *vs.* Group 4; and $p = 0.022$ for Group 1 *vs.* Group 5.

[c] $p = 0.003$ for Group 1 *vs.* Group 2; $p = 0.001$ for Group 1 *vs.* Group 3; $p = 0.003$ for Group 1 *vs.* Group 4; and $p = 0.013$ for Group 1 *vs.* Group 5.

$p < 0.05$ by one-way analysis of variance and Tukey tests.

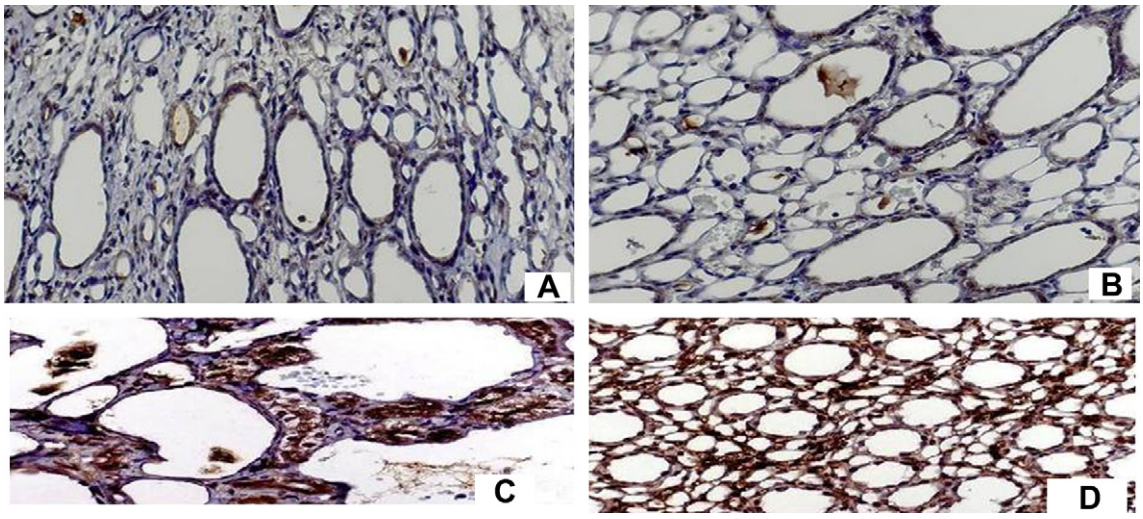

Figure 2. (A) Endothelial nitric oxide synthase (eNOS) expression in Group 2 and (B) inducible nitric oxide synthase (iNOS) expression in Group 3 were significantly decreased compared with those in Group 1. (C) eNOS and (D) iNOS (immunohistochemical stained method 40×).

muscles. The inhibition of PDE5 results in smooth muscle relaxation. PDE5 inhibitors also increase NO level in tissue through PDE5 inhibition. Many of the biological actions of NO are mediated by cyclic 3'5' guanosine monophosphate, which is rapidly degraded by PDEs [21]. Thus inhibiting PDEs elevates NO level in the tissue. In this study, we observed significant decreases of mean NOS levels in the renal tubular tissue after PDE5 inhibitor administration. In our opinion, this reduction is probably the result of the suppression of the elevated NO level in renal tissue. There are also some reports suggesting that sildenafil and other PDE5 inhibitors may have anti-inflammatory properties through inhibition of reactive oxygen species [22,23]. This effect can also explain the favorable findings obtained with the use of PDE5 inhibitors in this study.

The antiapoptotic properties of PDE5 inhibitors are still not well known. The antiapoptotic effect of PDE5 inhibitors was the main finding in our study. The possible mechanism of antiapoptotic effect of PDE5 inhibitors can be explained with sildenafil model. In different experimental studies, it has been suggested that the administration of sildenafil could result in opening of the mitochondrial adenosine triphospate (ATP)-sensitive potassium (mitoKATP) channels either directly or through a variety of signaling pathways, such as activation of protein kinase C and mitogen-activated protein kinases [24]. It has been shown that sildenafil may inhibit apoptosis by NO-mediated upregulation of Bcl-2/Bax ratio and attenuation of cytochrome C release [24].

Another finding in our study was the difference in affectivity of PDE5 inhibitors on tubular apoptosis. Although there were no differences between vardenafil and tadalafil groups, the mean apoptotic cell count was significantly decreased in the sildenafil group. As we know from the literature, PDE5 and PDE11 are commonly found in the kidneys, and tadalafil is more selective for PDE11 than sildenafil and vardenafil [25]. Therefore, it would not be a surprise to expect more favorable effects in the tadalafil group. Therefore, the additional improving effect of sildenafil may be explained with a probable stronger antiapoptotic effect of this drug. However, we could not evaluate the apoptosis with cell culture or more biomarkers because of the technical properties of our pathology laboratory; hence, it could be the restrictive factor of this study.

In conclusion, our findings demonstrate that PUUO increases renal tubular cell apoptosis and elevates NOS concentrations in renal tubular tissues after PUUO. PDE5 inhibitors, which are known to be effective in ED treatment, have a protective effect against the tubular apoptosis. In our opinion, these findings may be important in some clinical conditions, such as ureteral stones. Our findings that will be supported with the additional experimental studies may help physicians to use PDE5 inhibitors in the treatment of PUUO-related clinical conditions.

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